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FOREWORD

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I. INTRODUCTION

Current evidence suggests that breast carcinoma cells invade local tissues and metastasize by i) altering their cell-cell and cell-matrix interactions, ii) displaying an aberrant motile phenotype, and iii) either synthesizing, or inducing the synthesis of, proteolytic enzymes that degrade the structural barriers established by the extracellular matrix¹⁻³. The complex changes in the gene program of neoplastic cells that regulate the expression of this phenotype are largely undefined, but increased interest has focused on identifying those genes that are specifically overexpressed in human breast cancer^(e.g., 3-10). Such information not only provides new insights into the cellular factors that control tissue-invasive behavior, but may also lead to improvements in patient diagnosis and to the more rational design of therapeutic interventions³⁻¹⁰. Consistent with this rationale, direct comparisons of the gene expression profile displayed in normal versus neoplastic breast cancer cell lines, or normal and carcinomatous breast tissues, have provided a number of novel insights into the mechanisms and processes underlying tumor progression⁶⁻¹¹. Interestingly, despite the power of the analytical techniques employed for these purposes, the number of differentially expressed genes identified thus far are - at first glance - perplexingly small, despite the striking changes known to occur in cellular behavior^(e.g., 7,8). However, analyses of breast cancer cell lines grown *in vitro* or static tumor masses recovered from *in vivo* sites of disease may be problematic. First, comparisons between normal and neoplastic breast cancer cell lines grown atop plastic substrata *in vitro* will not recapitulate the complex interactions known to occur across the carcinoma-mesenchymal cell axis *in vivo*^{1,2}. Indeed, many of the most interesting gene products that have been associated with the expression of tissue-invasive phenotypes in breast cancer tissue are synthesized by surrounding stromal cells rather than the tumor itself^{2,3,10}. Secondly, while the gene expression patterns identified in tissues recovered from *in vivo* sites clearly circumvent the limitations inherent in the *in vitro* studies, only a small percentage of the cells recovered from a tumor mass at a single, fixed time point would be expected to be actively engaged in invasive behavior. Given the many similarities between developmental/tissue repair processes and malignant growth (re; the ability of cancer cells inappropriately recapitulate developmental programs associated with epithelial-mesenchymal cell transitions or repair programs associated with wound healing^{12,13}), we have considered the possibility that the *in situ* induction of a synchronous matrix remodeling program in normal tissues would allow for the more efficient isolation of those gene products critical to cancer cell invasion. Indeed, recent studies have demonstrated that gene expression patterns associated with the tissue remodeling program induced during the involution of the normal lactating mammary gland bear considerable overlap with those detected in the early stages of carcinogenesis (e.g., stromelysin-1, stromelysin-3, urokinase-type plasminogen activator, tissue inhibitor of metalloproteinases¹⁴⁻¹⁶). Hence, we propose to use the involuting mammary gland explant model as a means to rapidly enrich for, and identify, the subset of genes that control the disassembly of the extracellular matrix in cancerous states. Furthermore, by selectively identifying the subset of gene products that encode secreted proteins in breast cancer tissue, new diagnostics as well as novel targets for therapeutic intervention can be rapidly identified.

II. BODY

To begin characterizing those gene products that are induced in the involuting mammary gland, differentially expressed genes are being isolated by a subtractive hybridization protocol

established in our laboratory. Because most subtractive hybridization schemes require prohibitive amounts of poly(A)⁺ mRNA, we have opted to employ an alternative approach recently outlined by Lin et al wherein both driver (i.e., "control") and target (i.e., "test") cDNA libraries are constructed in phagemid vectors. Subsequently, one of the cDNA libraries is used to prepare single-stranded (ss) DNA, and the other to produce complementary biotinylated RNA by *in vitro* transcription. By subtracting lactating (wherein the expression/synthesis of matrix-degrading proteinases is minimal^{15,16}) from involuting explants, panels of induced genes will be isolated. [We note that involuting cultures can likewise be subtracted from lactating explants in order to isolate repressed transcripts.]

To first prepare the relevant RNAs, lactating and involuting mammary gland explant cultures were prepared according to the technique originally described by Banerjee et al. as modified by Young¹⁷. In brief, 3-4 week-old BALB/C mice were injected subcutaneously for nine consecutive days with progesterone (1 mg) and estradiol (1 µg). The second and fourth pairs of thoracic glands were excised and cultivated for nine days as whole organs on perforated cyclopore membranes inserted into wells of culture plates in Waymouth's medium in an atmosphere of 50% O₂/5% CO₂. The mammary glands were then cultured in serum-free media supplemented with aldosterone, ovine prolactin, insulin and hydrocortisone (APIH medium) to induce lobuloalveolar development. Involution was then initiated by withdrawing the lactogenic hormones (i.e., aldosterone, hydrocortisone and prolactin)¹⁸.

To begin to isolate the differentially expressed genes (i.e., between "control" lactating glands and "test" involuting tissues), poly(A)⁺ mRNA was isolated from control and test tissues using the FasTrack isolation kit. Test and control libraries have been constructed using the SuperScript plasmid system (Life Technologies) and the respective cDNAs ligated to pSPORT1 and pSPORT2 plasmid as described¹⁹. The plasmids were then electroporated (ElectroMaxTM DH125 cells) and dilutions of the transformed cells plated.

To isolate the differentially expressed genes, ssDNA and biotinylated RNA have been generated as described by Li et al¹⁹. These samples are now being prepared for the first round of subtractive hybridization.

III. CONCLUSIONS

Work has proceeded as planned to begin isolating differentially expressed gene products. Coincident with these experiments, conditioned media is being prepared from the involuting tissues in an attempt to generate polyclonal antisera against secreted proteins.

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